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### **Variation in tumor necrosis factor gene is not associated with susceptibility to COPD.**

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Ligation of both TLR2 and 9 on human cells has been shown to be associated with the development of T-reg cells and/or tolerance [5, 6]. We found that HSV infection conferred protection against atopy among Finnish children only. Besides the fact that atopy is infrequent in Russian Karelia, the overall exposure to microorganisms is overwhelming and the impact of saprophytes and other pathogens may well override the possible effects of HSV on the Russian side.

In summary, in an area with a relatively low microbial burden such as Finnish Karelia, herpes simplex virus appears to be able to exert immunomodulatory potential, which may have implications for the occurrence of atopy. This finding confirms the result shown previously in two other Western populations [7, 8].

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**STATEMENT OF INTEREST**  
None declared.

### REFERENCES

- 1 Mills KH. Regulatory T cells: friend or foe in immunity to infection? *Nat Rev Immunol* 2004; 4: 841–855.
- 2 Pekkarinen PT, von Hertzen L, Laatikainen T, *et al.* A disparity in the association of asthma, rhinitis, and eczema with allergen-specific IgE between Finnish and Russian Karelia *Allergy* 2007; 62: 281–287.
- 3 Suvas S, Azkur AK, Kim BS, Kumaraguru U, Rouse BT. CD4+CD25+ regulatory T cells control the severity of viral immunoinflammatory lesions *J Immunol* 2004; 172: 4123–4132.
- 4 Herbst-Kralovetz M, Pyles R. Toll-like receptors, innate immunity and HSV pathogenesis *Herpes* 2006; 13: 37–41.
- 5 Taylor RC, Richmond P, Upham JW. Toll-like receptor 2 ligands inhibit Th2 responses to mite allergens *J Allergy Clin Immunol* 2006; 117: 1148–1154.
- 6 Moseman EA, Liang X, Dawson AJ, *et al.* Human plasmacytoid dendritic cells activated by CpG oligodeoxynucleotides induce the generation of CD4+CD25+ regulatory T cells *J Immunol* 2004; 173: 4433–4442.
- 7 Matricardi PM, Rosmini F, Panetta V, Ferrigno L, Bonini S. Hay fever and asthma in relation to markers of infection in the United States *J Allergy Clin Immunol* 2002; 110: 381–387.
- 8 Illi S, von Mutius E, Lau S, *et al.* Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study *BMJ* 2001; 322: 390–395.

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# Variation in the tumour necrosis factor gene is not associated with susceptibility to COPD

To the Editors:

In a recent issue of the *European Respiratory Journal*, TANAKA *et al.* [1] studied polymorphisms in the tumour necrosis factor (TNF) and lymphotoxin A genes with respect to their effect on lung function of smokers, and failed to find any association with chronic obstructive pulmonary disease (COPD) phenotypes. TANAKA *et al.* [1] acknowledge that their work is not a true case–control study, but that it would be better described as an investigation of genetic contribution to disease severity. There have been several studies of variation in TNF with respect to susceptibility to COPD, although many of these have used relatively small sample sizes and are therefore underpowered, and so are likely to lead to results that cannot be replicated.

As part of a European Union collaborative project, we have studied polymorphisms within the TNF gene in a large collection of well-characterised Caucasian COPD patients (n=1,018) and control subjects (n=911). COPD cases and

TABLE 1 Frequency of single nucleotide polymorphisms (SNPs) genotyped in the tumour necrosis factor gene#		
	COPD patients	Controls
rs1799964* (T-1031C)	0.207	0.193
rs1800629* (G-308A)	0.179	0.178
rs361525* (G-238A)	0.055	0.049
rs1800610 (G489A)	0.096	0.096
rs3093662 (A851G)	0.084	0.077
rs1800628 (G3512A)	0.127	0.119

COPD: chronic obstructive pulmonary disease. #: numbering with respect to the transcription start site is shown in parentheses; \*: SNPs studied by TANAKA *et al.* [1].

**TABLE 2** Haplotypes of tumour necrosis factor single nucleotide polymorphisms and frequency in cases and controls

	Polymorphism						Frequency	
	rs1799964	rs1800629	rs361525	rs1800610	rs3093662	rs1800628	Controls	COPD cases
1	T	A	G	G	A	G	0.058	0.052
2	T	G	G	G	A	G	0.502	0.489
3	T	G	G	A	A	G	0.095	0.095
4	T	G	G	G	G	G	0.028	0.029
5	A	G	G	G	A	G	0.146	0.153
6	A	G	A	G	G	G	0.050	0.053
7	T	A	G	G	A	A	0.120	0.127
Rare haplotypes							0.001	0.002

COPD: chronic obstructive pulmonary disease.

control subjects were recruited from six European centres, as previously reported [2]. The characteristics of each group are summarised as follows, with data expressed as mean  $\pm$  SD, where appropriate. Controls: 63.5% male; age  $60.8 \pm 8.9$  yrs; smoking history  $38.6 \pm 17.4$  pack-yrs; forced expiratory volume in one second (FEV<sub>1</sub>)  $95.3 \pm 10.9\%$  predicted; FEV<sub>1</sub>/forced vital capacity (FVC)  $77.9 \pm 4.9\%$ . COPD cases: 69.6% male; age  $65.8 \pm 8.2$  yrs; smoking history  $48.9 \pm 23.6$  pack-yrs; FEV<sub>1</sub>  $43.0 \pm 15.3\%$  pred; FEV<sub>1</sub>/FVC  $47.5 \pm 12.2\%$ .

Six single nucleotide polymorphisms (SNPs) in TNF (table 1) were genotyped using Taqman® probes (Geneservice Ltd, Babraham, UK). Primer and probe sequences are available on request. As quality control measures for the genotyping, 2% of samples of known genotype were included and 10% of samples were present in duplicate to check for concordance. All SNPs were in Hardy–Weinberg equilibrium.

The association of single SNPs with COPD was carried out using a Chi-squared analysis; none of the SNPs showed a significant difference in genotype frequency between cases and controls ( $p \geq 0.331$ ). Linear regression analysis in cases and controls was used to identify any possible effect of TNF genotype on FEV<sub>1</sub> (with age, smoking history and sex as covariates). Using a stringent cut-off, this also failed to find any significant effect, with the lowest p-value being obtained for rs1800628 in controls only  $p=0.010$ . Allowing for multiple testing, this is unlikely to be a true association.

A case–control analysis of TNF haplotypes was carried out according to [3]. Haplotypes of the TNF SNPs are shown in table 2. This analysis identified a total of 10 haplotypes, seven of which were present at a frequency  $>2\%$ . There was no significant difference in the frequency of these haplotypes between COPD cases and controls (global score statistic=2.024, 7 degrees of freedom;  $p=0.959$ ). Similarly, using the omnibus test performed over all haplotypes in the SAS procedure PROC HAPLOTYPED [4], none of the possible subsets of the six SNPs showed a significant relationship with COPD.

In this large case–control study using well-characterised COPD patients and controls, we failed to find any association of the TNF polymorphisms with the development of COPD. This is in

agreement with other smaller studies in Caucasian populations [5–8], although many studies have only considered the -308 SNP. One study, using 169 Caucasian COPD patients and 358 controls, showed an increase of the 489\_GA genotype in the COPD patients with an associated odds ratio of 1.9 [9]; however, this is not replicated in the current study, suggesting that it may have been a false-positive result.

For allele frequencies ranging 0.05–0.20 (using a dominant model and  $\alpha=0.01$ ), the current study has  $>80\%$  power to detect minimum effect sizes of 1.4–1.65. The fact that we see no association with any of the tumour necrosis factor single nucleotide polymorphisms or haplotypes makes it highly unlikely that polymorphisms in this gene play a major role in the susceptibility to chronic obstructive pulmonary disease.

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#### STATEMENT OF INTEREST

None declared.

## REFERENCES

- 1 Tanaka G, Sandford AJ, Burkett K, *et al.* Tumour necrosis factor and lymphotoxin A polymorphisms and lung function in smokers *Eur Respir J* 2007; 29: 34–41.
- 2 Chappell S, Daly L, Morgan K, *et al.* Cryptic haplotypes of SERPINA1 confer susceptibility to chronic obstructive pulmonary disease *Hum Mutat* 2006; 27: 103–109.
- 3 Sinnwell JP, Schaid DJ, Yu Z. Statistical analysis of haplotypes. Version 1.2.2., <http://cran.r-project.org/src/contrib/Descriptions/haplo.stats.html> Date last update: June 2007.
- 4 SAS/Genetics User's Guide. Release 8.2. Cary, SAS Institute Inc., 2002.
- 5 Hersh CP, DeMeo DL, Lange C, *et al.* Attempted replication of reported chronic obstructive pulmonary disease candidate gene associations *Am J Respir Cell Mol Biol* 2005; 33: 71–78.
- 6 Higham MA, Pride NB, Alikhan A, Morrell NW. Tumour necrosis factor- $\alpha$  gene promoter polymorphism in chronic obstructive pulmonary disease *Eur Respir J* 2000; 15: 281–284.
- 7 Patuzzo C, Gilè LS, Zorzetto M, *et al.* Tumor necrosis factor gene complex in COPD and disseminated bronchiectasis *Chest* 2000; 117: 1353–1358.
- 8 Ruse CE, Hill MC, Tobin M, *et al.* Tumour necrosis factor gene complex polymorphisms in chronic obstructive pulmonary disease *Respir Med* 2007; 101: 340–344.
- 9 Küçükaycan M, Van Krugten M, Pennings HJ, *et al.* Tumor necrosis factor- $\alpha$  +489G/A gene polymorphism is associated with chronic obstructive pulmonary disease *Respir Res* 2002; 3: 29.

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# Beyond the “ABC approach”

To the Editors:

I read with interest the recent review by CELLI and BARNES [1], wherein the authors have beautifully discussed the aetiology, cellular and molecular mechanisms, management and new definition of exacerbations of chronic obstructive pulmonary disease (COPD).

In the management of acute exacerbations of COPD they have discussed the role of three classes of drugs (antibiotics, bronchodilators and corticosteroids, *i.e.* an “ABC approach”); however, the role of oxygen therapy, theophylline and supportive management is not mentioned. All of these have implications for the management of the disease.

Hypoxia at exacerbation of COPD is quite common and may be life threatening. The importance of controlled oxygen therapy during the management of acute exacerbation cannot be overlooked [2].

Theophylline is widely used in the management of stable patients with COPD. A recent meta-analysis evaluating the role of intravenous aminophylline in cases of acute exacerbation of COPD did not find any beneficial effect in terms of improvement of pulmonary function or symptoms [3]. However, the withdrawal of methylxanthines in patients already receiving them can worsen lung function, clinical status, exercise performance and ratings of dyspnoea [4]. Therefore, patients already receiving oral preparations of methylxanthines for stable disease should continue on such treatments at the time of exacerbation. The current Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines even suggest adding aminophylline to standard therapy for patients with moderate-to-severe exacerbations or those not responding to nebulised short-acting bronchodilators [5].

Appropriate fluid balance (with special attention to the administration of diuretics), nutritional aspects, anticoagulants,

and cardiovascular agents are the most complementary standard measures. Manual or mechanical chest percussion and postural drainage may be beneficial in patients producing >25 mL sputum per day or with lobar atelectasis.

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## STATEMENT OF INTEREST

None declared.

## REFERENCES

- 1 Celli BR, Barnes PJ. Exacerbations of chronic obstructive pulmonary disease *Eur Respir J* 2007; 29: 1224–1238.
- 2 Aubier M, Murciano D, Milic-Emili J, *et al.* Effects of the administration of O<sub>2</sub> on ventilation and blood gases in patients with chronic obstructive pulmonary disease during acute respiratory failure *Am Rev Respir Dis* 1980; 122: 747–754.
- 3 Barr RG, Rowe BH, Camargo CA Jr. Methylxanthines for exacerbations of chronic obstructive pulmonary disease: meta-analysis of randomised trials *BMJ* 2003; 327: 643.
- 4 Kirsten DK, Wegner RE, Jörres RA, Magnussen H. Effects of theophylline withdrawal in severe chronic obstructive pulmonary disease *Chest* 1993; 104: 1101–1107.
- 5 Rabe KF, Hurd S, Anzueto A, *et al.* Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease–2006 Update. *Am J Respir Crit Care Med* 2007; [Epub ahead of print PMID: 17507545].

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